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Urinary Organophosphate Metabolite Concentrations and Pregnancy Outcomes among Women Conceiving through *in Vitro* Fertilization in Shanghai, China

Peipei Hu,[1] Angela Vinturache,[2*] Hong Li,[3*] Ying Tian,[4,5] Lei Yuan,[4] Chen Cai,[1] Min Lu,[1] Jiuru Zhao,[6,7] Qianqian Zhang,[6,7] Yu Gao,[4] Zhiwei Liu,[8] and Guodong Ding[1]*

[1]Department of Respiratory Medicine, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, China
[2]Department of Obstetrics & Gynecology, Queen Elizabeth II Hospital, Alberta, Canada
[3]Departments of Nursing, International Peace Maternity and Children Hospital of China Welfare Institution, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[4]Department of Environmental Health, School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[5]MOE-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[6]International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[7]Shanghai Key Laboratory of Embryo Original Disease, Shanghai, China
[8]Departments of Neonatology, International Peace Maternity and Children Hospital of China Welfare Institution, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Background: Animal studies suggest that pesticide exposure elicits endocrine changes, increases embryo implantation failure, and decreases litter size. However, only a few epidemiological studies have evaluated the effects of pesticides on the outcomes of *in vitro* fertilization (IVF) pregnancies.

Objectives: This study examined the associations between preconception organophosphate pesticides (OP) exposure and pregnancy outcomes among women undergoing IVF in a Chinese population.

Methods: This study included 522 women with infertility who underwent IVF. Women were recruited from a prospective study, the China National Birth Cohort (CNBC), from Shanghai, China, between July 2017 and December 2018. Demographic and clinical information were collected from medical records and through questionnaires. Preconception exposure to OP was assessed by measuring six nonspecific dialkylphosphate (DAP) metabolites [diethylthiophosphate (DETP), diethylphosphate (DEP), diethyldithiophosphate (DEDTP), dimethylthiophosphate (DMTP), dimethylphosphate (DMP), dimethyldithiophosphate (DMDTP)] in urine samples collected at recruitment. Generalized estimating equation (GEE) models were used to evaluate the associations between OP and pregnancy outcomes.

Results: Compared with women in the lowest quartile (Q_1) of individual DEP and Σ_4DAP (the sum of DMP, DMTP, DEP, and DETP), women in the highest quartile (Q_4) had lower odds of successful implantation, clinical pregnancy, and live birth, and most of the negative trends were significant (p-trends <0.05). There were no significant associations between urinary DAP concentrations and early IVF outcomes, including total and mature oocyte counts, best embryo quality, fertilization, E_2 trigger levels, and endometrial wall thickness.

Conclusion: Preconception OP exposure was inversely associated with successful implantation, clinical pregnancy, and live birth in women who underwent IVF. https://doi.org/10.1289/EHP7076

Introduction

One in every four couples in developing countries and an estimated 15%–20% of women of childbearing age in China are affected by infertility (WHO; Qiao and Feng 2014). This percentage is likely to rise with the trend of delayed childbearing (Qiao and Feng 2014). Assisted reproduction technologies (ART), including *in vitro* fertilization (IVF), has had a tremendous impact on the treatment of infertility. Approximately 700,000 ART treatments are performed in China annually (Yu et al. 2018). The success rate of ART, expressed as pregnancy rate per ART cycle and live birth delivery rate, is still ~30% (Liu and Wang 2017). The high prevalence of infertility and the relatively low success rates of ART treatment point to the importance of identifying potential risk factors that may hinder the success of the ART. Among these factors, environmental pollutants, unhealthy lifestyles, and work-related stress have been identified as potential contributors (Homan et al. 2007; Xu et al. 2017; Younglai et al. 2005). However, environmental pollutants such as pesticides have attracted international attention and recently have been regarded as possible contributors to suboptimal or even adverse reproductive outcomes (Levario-Carrillo et al. 2004; Pastore et al. 1997; Zhu et al. 2006).

Organophosphate pesticides (OP) are one of the most commonly used classes of insecticides, with an annual usage of 70,000 metric tons in 2015, contributing to approximately one-quarter of all insecticides (about 300,000 metric tons) used throughout China (Shu et al. 2014, 2016). Human exposure to OP occurs via consumption of contaminated water and food. Ubiquitous exposure to OP is a global public health issue (He et al. 2018). Our previous studies documented that exposure to OP was associated with prolonged time of pregnancy, decreased length of gestation, and poorer intellectual development in offspring (Hu et al. 2018; Wang et al. 2012; Wang et al. 2017b). Most studies examining the relationship between OP exposure and adverse reproductive outcomes have primarily focused on women who conceived naturally (Levario-Carrillo et al. 2004; Pastore et al. 1997; Zhu et al. 2006; Hu et al. 2018; Wang et al. 2012; Wang et al. 2017b). The evidence of adverse associations between OP and endocrine function raised further concerns about

*These authors contributed equally to this work.
Address correspondence to Guodong Ding, Department of Respiratory Medicine, Shanghai Children's Hospital, Shanghai Jiao Tong University, 1400 West Beijing Rd., Shanghai 200040, China. Telephone: +86-21-62791087. Email: dingguodong@shchildren.com.cn. Zhiwei Liu, Departments of Neonatology, International Peace Maternity and Children Hospital of China Welfare Institution, School of Medicine, Shanghai Jiao Tong University, 910 Hengshan Rd., Shanghai 200030, China. Telephone/Fax: +86-21-64073899. Email: liuzhiwei@hotmail.com. Yu Gao, Department of Environmental Health, School of Public Health, Shanghai Jiao Tong University School of Medicine, 280 South Chongqing Rd., Shanghai 200025, China. Telephone/Fax: +86-21-64663944. Email: gaoyu_ciel@sjtu.edu.cn

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chronic OP exposures and potential effects on pregnancy outcomes in women undergoing IVF.

Animal and *in vitro* studies suggested that exposure to OP disrupts reproductive function through inhibition of follicular growth, impaired gamete quality and oocyte maturation, and poor embryo development (Ding et al. 2011; Tian et al. 2000; Tian et al. 2009). Few epidemiological studies to date examined the association between pesticide exposures and reproductive outcomes in women who sought ART, showing inconsistent results (Chiu et al. 2018; Jirsová et al. 2010; Mahalingaiah et al. 2012; Al-Saleh et al. 2009; Al-Hussaini et al. 2018; Bloom et al. 2017). Therefore, the present study used data from the China National Birth Cohort (CNBC) prospective study to explore the association between preconception OP exposure and reproductive health outcomes among women undergoing IVF.

Methods

Study Population

The population of this study was drawn from the CNBC Study, an ongoing prospective birth cohort that has as a main objective to evaluate the effects of ART on maternal and child health outcomes. The recruitment for the cohort started in 2016 and is ongoing. Briefly, the study recruits couples ≥20 years of age seeking fertility evaluation and treatment at the Center for Reproductive Medicine at the International Peace Maternity and Child Health Hospital, Shanghai, China. The couples are eligible for ART if the women have not become pregnant after a year or more of unprotected intercourse. The study prospectively collects self-reported questionnaire data, biological samples (e.g., follicular fluid, semen, blood, urine), and medical information abstracted from fertility clinic and hospital records.

For the purpose of the present study, the study population was restricted to women who underwent IVF at the Center for Reproductive Medicine between July 2017 and December 2018. Women considered eligible were Shanghai residents, had no plan to move out of Shanghai in the following 5 years, had contributed their own oocytes to the IVF cycles, and planned to give birth in our hospital. Women who used cryo-thawed or donor oocytes were not eligible for enrollment. After recruitment, the women were followed throughout each of their IVF treatment cycles until either a live birth was achieved or the treatment at the Center for Reproductive Medicine was discontinued. From 597 women who met the eligibility criteria, 522 women were included in the study (Figure 1).

The study protocol was approved by the Medical Ethics Committee of International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine (GKLW-2016-21). Each participant in the study signed an informed consent form.

Questionnaires

At recruitment, trained research staff administered a 30-min standardized questionnaire that included questions on demographic characteristics, lifestyle factors, reproductive medical history, and common environmental pollutant exposure. In addition, women were asked to complete a self-reported standardized Perceived Stress Scale (PSS-10). The reliability and validity of the PSS-10 has been reported elsewhere (Leung et al. 2010; Nordin and Nordin 2013; Dambi et al. 2018). The psychometric robustness of the Chinese version of the PSS-10 has been previously assessed (Lu et al. 2017; Leung et al. 2010).

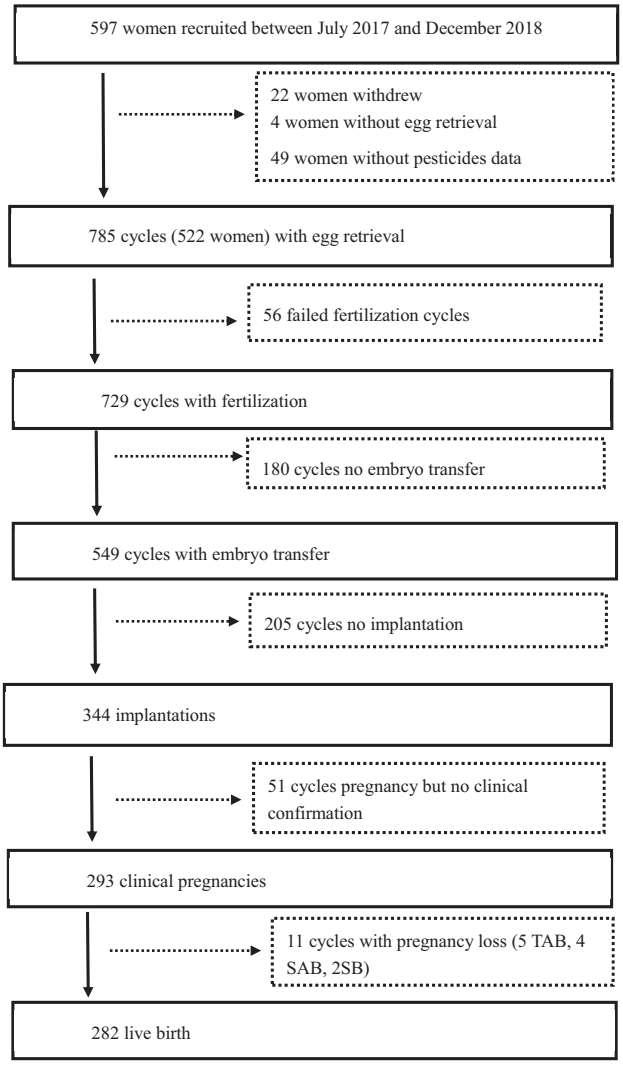

Figure 1. Participants' selection flowchart. Note: SAB, spontaneous abortion; SB, stillbirth; TAB, therapeutic abortion.

Biological Specimens

Approximately 50 mL single spot urine samples (not necessarily a fasting sample) were collected from women at enrollment and then aliquoted to 10 mL polypropylene tubes and stored at −80°C until further analysis. Measurements of nonspecific dialkylphosphate (DAP) metabolites of OP were conducted at Shanghai Clinical Research Center using gas chromatography–tandem mass spectrometry (GC-MS) (Ueyama et al. 2010). Six analytes were measured in each specimen: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Almost all OP are metabolized to at least one of six possible DAP metabolites, as shown in Table S1 (Duggan et al. 2003).

The limits of detection (LOD) were 0.18 μg/L for DMP, 0.3 μg/L for DMTP and DMDTP, 0.06 μg/L for DEP and DETP, and 0.09 μg/L for DEDTP, respectively. Metabolite concentrations below the LOD were replaced with LOD/$\sqrt{2}$ (Hornung and Reed 1990). The molar concentrations of DMP, DMTP, DEP, and DETP were summed to derive Σ_4DAP as a summary measure of environmental OP exposures (Arcury et al. 2006).

Quality control (QC) samples consisted of urine blanks and urine spikes. Urine blanks were control samples of pooled urine

from eight healthy adult volunteers. Two types of urine spikes control samples, high and low DAP concentrations, were prepared by adding predetermined high vs. low concentrations of six individual DAP metabolites to urine blanks. QC samples and urine samples were measured simultaneously. One urine blank and 4 urine spikes (2 duplicate samples for high and low concentrations) were included at every 38 urine samples. The percent of relative standard deviation (% RSD) for DAP metabolites ranged from 0.5% to 6.3% for the within-run precision, and from 3.0% to 17.8% for the between-run precision, as described elsewhere (Wang et al. 2017b).

Metabolite concentrations were normalized to creatinine (Cr) concentrations in urine to correct for variable urine dilutions in the spot urine samples analyses. Cr concentrations in urine were measured with an automated chemistry analyzer (7100 Hitachi).

Clinical Data and IVF Outcomes

Clinical information on follicle stimulating hormone (FSH) and serum peak estradiol (E_2) concentrations, endometrial thickness, and IVF outcomes was abstracted from the patients' electronic medical records at the entry in the study and after each IVF cycle. FSH was measured in serum obtained on day 3 of the menstrual cycle. Peak E_2 concentration, defined as the highest level of E_2 prior to oocyte retrieval, was obtained on the day of ovulation trigger with exogenous human chorionic gonadotropin (HCG). Endometrial thickness (millimeters) was measured by transvaginal ultrasound scan 2 d before egg retrieval.

Briefly, the IVF process can be divided into four steps: the start of controlled ovarian stimulation, oocyte retrieval, embryo transfer, and pregnancy test. At each IVF cycle, women undergo different ovarian stimulation protocols based on their age and infertility diagnosis, including super long gonadotropin-releasing hormone (GnRH) agonist, standard long GnRH agonist, short GnRH agonist, GnRH antagonist, mild stimulation, and natural cycle (Shrestha et al. 2015). Because in our study most women were of advanced age and/or had a diminished ovarian reserve, the GnRH antagonist protocol was most used. In this protocol, fertilization treatment started with daily injection of gonadotrophins, the dose of which was individualized for the women's age, ovarian response, and the result of ovarian reserve test. After 4–5 d of ovarian follicle stimulation, ultrasound and daily serum estradiol measurements were performed to assess and confirm uterine lining development and ovarian follicles' maturation. A dose of 0.25 mg of GnRH antagonist Cetrorelix Acetate (Cetrotide®; Merck-Serono) was started on the day when at least one follicle reached ≥12–13 mm in diameter and was continued until recombinant HCG (Ovidrel®; Merck-Serono) was administered. When at least 2–3 follicles reached 18 mm in size, the recombinant HCG was administered to trigger the maturation of follicles. Oocyte retrieval was completed under transvaginal ultrasonographic guidance, 34–36 h after HCG injection. After egg retrieval, oocytes were counted and classified as germinal vesicle, metaphase I, metaphase II (MII), or degenerated (Chian et al. 2004). Fertilization was confirmed 17–20 h after insemination by the presence of a fertilized oocyte with two pronuclei (2PN). Main variables in assessment of embryo included the cleavage rate, equality of blastomeres, the degree of fragmentation, and mononuclearity in blastomeres. The best quality embryo was defined as ≥4 cells on day 2 or ≥6 cells on day 3, fragmentation <20%, and all blastomeres equal or almost equal in size (Nasiri and Eftekhari-Yazdi 2015). The intrauterine transfer occurred on day 2, 3, or 5 of embryo maturation in culture; most transfers included ≤2 best-quality embryos on day 3.

Pregnancy outcomes were assessed in all women who underwent an embryo transfer. Implantation was defined as positive serum β-hCG level on day 14 after embryo transfer, and clinical pregnancy was confirmed by the presence of a gestational sac on the ultrasound performed 3–4 wk after positive hCG test. Live birth was defined as live-born neonate on or after 28 wk gestation.

Statistical Analysis

Descriptive statistics summarized demographic characteristics, clinical outcomes, and DAP metabolites concentration. Demographic and clinical characteristics of the study participants were reported using mean values ± standard deviation (SD) or percentages as appropriate. The four DAP metabolites concentrations were categorized into quartiles based on the Cr-adjusted concentrations due to detectable frequencies of most DAP metabolites (DMTP, DEP, DETP) ≥80%. However, 31% of DMP metabolite concentrations were lower than LOD and were assigned a value equivalent to the $\mathrm{LOD}/\sqrt{2}$ and then divided by urinary Cr levels. Due to relatively low detection rate of DMDTP (13.8%) and DEDTP (1.9%), these two metabolites were not included in further statistical analyses. Tests for trend (p-trend) were performed by modeling an ordinal variable coded as the median concentration for each quartile.

Potential confounders were selected from previous studies of environmental pollutant exposures on IVF outcomes (Messerlian et al. 2018; Carignan et al. 2017; Messerlian et al. 2016). The following covariates were included in all models: age, body mass index (BMI), duration of infertility, smoking status, education, annual household income, and infertility diagnosis. In addition, we adjusted for two variables based on the PSS-10 questions that were associated with two or more IVF outcomes in bivariate analysis ($p < 0.10$): "Have you been upset because of something that happened unexpectedly?" and "Have you felt unable to control the important things in your life?". The responses were categorized into three groups: never or almost never, sometimes, and fairly often or very often.

To evaluate the association between the urinary DAP metabolites concentrations and IVF outcomes, we used generalized estimating equations (GEE) models to account for multiple IVF cycles and end-point outcomes in the same women (Chiu et al. 2018, Ehrlich et al. 2012; Machtinger et al. 2013; Mok-Lin et al. 2010). The normal distribution and identity link were applied for continuous outcomes (peak E_2 concentration, endometrial thickness), and a binomial distribution and logit link function were specified for fertilization and clinical outcomes (implantation, clinical pregnancy, and live birth) (Machtinger et al. 2013; Messerlian et al. 2018). Finally, Poisson regression models using a GEE approach were applied for the total number of oocytes and mature MII oocytes as well as best quality embryo (Ehrlich et al. 2012; Mok-Lin et al. 2010).

In view of the multiple testing, we adjusted the p-values using Benjamini-Hochberg (BH) procedure to control false discovery rate (FDR) at <5% (Bender and Lange 2001; Benjamini and Hochberg 1995).

In addition, to facilitate comparisons for the above-mentioned three clinical outcomes with results from other similar studies, we also restricted the sensitivity analysis to the first IVF cycle only (Carignan et al. 2017). We further examined the associations between Σ_3DAP (the sum of DMP, DMTP, and DETP) and DEP concentrations with clinical outcomes to determine whether our results could be attributed to DEP rather than any other urinary DAP metabolite.

To compare the outcomes of insemination techniques including IVF and intracytoplasmic sperm injection (ICSI) on the number of MII oocytes and fertilization, we stratified analyses for MII oocytes and fertilization by insemination type, IVF vs. ICSI.

To explore potential effect modification by a diagnosis of infertility, we also stratified analyses by cause of infertility, male vs. female factor.

Stratified analyses were also conducted to explore whether women's age (<35 y vs. ≥35 y) and BMI (<24 kg/m^2 vs. ≥24 kg/m^2), two well-known predictors for female fertility, modified the relationship between urinary DAP concentrations and clinical outcomes (Steiner and Jukic 2016; Luke et al. 2011). Potential interactions between women's age or BMI and OP were tested by adding a cross product term (exposure × age or exposure × BMI) in our models, with *p*-value for the interaction term of <0.1 being considered significant.

All statistical analyses were performed using SPSS 19.0 software (SPSS Inc.); $p < 0.05$ (two-tailed) was considered statistically significant.

Results

The demographic characteristics of the 522 participants included in the study are presented in Table 1. The average age was 33.56 ± 4.41 years of age, with an average BMI of 22.12 ± 2.96 kg/m^2. Women had on average 3.45 ± 2.31 y of infertility at the time of enrollment. Approximately half of the women (51.0%) had an educational attainment of bachelor's degree and reported an annual household income ≥200,000 CNY (54.6%). The vast majority of women (96.4%) never smoked. In 61.5% of infertility diagnoses, a female cause including ovulatory, tubal, endometrial, or uterine factors, was involved. Regarding the PSS-10 questionnaire, approximately two-thirds (67.0%) of the women responded, "Sometimes," or "Fairly often or very often" to the question, "Have you been upset because of something that happened unexpectedly?"; in addition, 42.5% yielded the same answers to the question, "Have you felt unable to control the important things in your life?".

The clinical characteristics and cycle outcomes are summarized in Table 2. The long GnRH agonist and GnRH antagonist were the primary stimulation protocols used in the clinic. GnRH antagonist contributed to 60% of the treatment cycles. The 522 women contributed a total of 785 IVF cycles (mean 1.5 cycles per woman), with 70%, 18%, and 12% contributing 1, 2, and 3–6 cycles, respectively, and 1 who contributed 9 cycles (Table S2). Of the 785 IVF cycles, 327 (41.7%) cycles were ICSI oocyte insemination, with the rest of 458 (58.3%) being IVF embryo transfer. Mean ± SD day 3 FSH (IU/L), and peak estradiol (pg/mL) concentrations were 7.73 ± 2.67 and 9,475.36 ± 6,274.76, respectively. The total number of oocytes retrieved ranged from 1 to 43, with a mean ± SD of 9.48 ± 7.66. Of the transfers, 21% were single embryo transfers, and 50.3% of the transfers were on day 3, at the cleavage stage (Table 2).

An overview of the 785 IVF cycles is shown in Figure 1. In brief, 549 cycles of the initial 785 cycles were embryo transfers

Table 1. Demographic characteristics of the 522 women included in the study.

Characteristic	Mean ± SD (range) or *n* (%)
Maternal age (y)	33.56 ± 4.41 (23.00–47.00)
BMI (kg/m^2)	22.12 ± 2.96 (15.63–32.47)
Duration of infertility (y)	3.45 ± 2.31 (1.50–16.00)
Prepregnancy age (y)	
<35	316 (60.5)
≥35	206 (39.5)
Prepregnancy BMI (kg/m^2)	
<24	404 (77.4)
≥24	118 (22.6)
Education	
<College graduate	185 (35.4)
College graduate	266 (51.0)
>College graduate	71 (13.6)
Annual household income (CNY)	
<200,000	237 (45.4)
200,000–300,000	139 (26.6)
>300,000	146 (28.0)
Smoking	
Never smoker	503 (96.4)
Ever smoker	19 (3.7)
Current smoker	4 (0.8)
Former smoker	15 (2.9)
Infertility diagnosis	
Female factor	321 (61.5)
Male factor	196 (37.5)
Unexplained	5 (1.0)
Have you been upset because of something that happened unexpectedly?[a]	
Never or almost never	172 (33.0)
Sometimes	296 (56.7)
Fairly often or very often	54 (10.3)
Have you felt unable to control the important things in your life?[a]	
Never or almost never	300 (57.5)
Sometimes	201 (38.5)
Fairly often or very often	21 (4.0)

Note: BMI, body mass index; CNY, Chinese yuan; PSS, perceived stress scale.
[a]Items from the PSS-10 questionnaire.

Table 2. Treatment protocols, cycle-specific characteristics, and pregnancy outcomes from 785 *in vitro* fertilization cycles among 522 women.

Characteristics	Mean ± SD (range) or *n* (%)
Treatment protocol	
Long GnRH agonist protocol	145 (18.5)
Super long GnRH agonist	10 (1.3)
Short GnRH agonist	6 (0.8)
GnRH antagonist protocol	472 (60.1)
Mild stimulation	107 (13.6)
Natural cycle	45 (5.7)
Oocyte insemination technique	
IVF	458 (58.3)
ICSI	327 (41.7)
Embryo transfer day	
No embryos transferred	236 (30.1)
Day 2	93 (11.8)
Day 3	396 (50.4)
Day 5	60 (7.6)
Number of embryos transferred	
No embryos transferred	236 (30.1)
1 embryo	166 (21.1)
2 embryos	383 (48.8)
Controlled ovarian hyperstimulation outcomes	
Peak estradiol (pg/mL)	9475.36 ± 6274.76 (299.00–4307.00)
Day 3 FSH (IU/L)	7.73 ± 2.67 (2.10–23.70)
Total number of oocytes retrieved	9.48 ± 7.66 (1–43)
Mature (MII) oocytes retrieved	7.28 ± 5.82 (0–30)
Normal (2PN) fertilized oocytes	6.59 ± 5.59 (0–27)
Total embryos	6.36 ± 5.43 (0–27)
Best embryos	3.25 ± 3.33 (0–22)
Fertilization rate[a]	0.68 ± 0.28 (0–1)
Pregnancy outcomes	
Fertilization	729 (92.9)
Implantation failure[b]	441 (56.2)
Clinical pregnancy[c]	293 (37.4)
Spontaneous abortion	5 (0.6)
Therapeutic abortion	4 (0.5)
Stillbirth	2 (0.2)
Live birth[d]	282 (36.1)

Note: FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; IU, international unit; IVF, in vitro fertilization.
[a]Fertilization rate as the number of oocytes with 2PN divided by the number of retrieved oocytes.
[b]Implantation failure was defined as a negative pregnancy test (serum β-hCG level <6 mIU/mL) 14 d following embryo transfer or insemination.
[c]Clinical pregnancy was defined as the presence of an intrauterine gestational sac and fetal heartbeat confirmed by ultrasound by 6 wk of gestation.
[d]Live birth as the delivery of a live neonate on or after 28 wk gestation.

Table 3. Urinary concentrations of DAP metabolites ($n = 522$).

	Detection rate	Not adjusted for Cr (μg/L)				Cr-adjusted (μg/g)			
Metabolites	*n* (%)	25th	50th	75th	95th	25th	50th	75th	95th
DMP	360 (69.0)	<LOD	5.12	20.21	121.92	<LOD	5.36	19.58	123.72
DMTP	436 (83.5)	0.76	1.25	2.42	12.23	0.74	1.37	2.47	11.13
DEP	444 (85.1)	1.13	11.93	43.93	176.82	1.23	10.15	41.06	152.78
DETP	480 (92.0)	0.92	1.68	3.31	14.92	0.92	1.83	3.47	10.25
DMDTP	72 (13.8)	<LOD	<LOD	<LOD	0.76	<LOD	<LOD	<LOD	0.89
DEDTP	10 (1.9)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Σ_4DAP[a]	—	39.32	197.48	605.47	2050.50	47.45	216.67	567.58	1904.24

Note: The LOD were 0.18 μg/L for DMP, 0.3 μg/L for DMTP and DMDTP, 0.06 μg/L for DEP and DETP, and 0.09 μg/L for DEDTP. Cr, creatinine; DAP, dialkylphosphate; DEDTP, diethyldithiophosphate; DEP, diethylphosphate; DETP; diethylthiophosphate; DMDTP, dimethyldithiophosphate; DMP, dimethyl phosphate; DMTP, dimethylthiophosphate; LOD, limits of detection.
[a]Σ_4DAP was the summary of molar concentrations of DMP, DMTP, DEP, and DETP. The unit for DAPs (not adjusted for Cr) is nmol/L; the unit for DAP (adjusted for Cr) is nmol/g Cr.

(70%). Of the cycles that underwent embryo transfer, the percent resulting in implantation, clinical pregnancy, and live birth were 62.7%, 53.4%, and 51.3%, respectively.

The DAP metabolites concentrations in urine samples reported as percentiles (25th, 50th, 75th, and 95th), unadjusted and adjusted for Cr, are summarized in Table 3. Detection rates were sufficient for further analyses for DETP (92.0%), DEP (85.1%), DMTP (83.5%), and DMP (69.0%) but were low for DMDTP (13.8%) and DEDTP (1.9%). The Cr-adjusted median concentrations of urinary DMP, DMTP, DEP, and DETP were 5.36, 1.37, 10.15, and 1.83 μg/g Cr, respectively.

Distributions of DAP metabolites concentrations according to age (<35 or ≥35 years of age) and BMI (<24 or ≥24 kg/m^2) are presented in Tables S3 and S4. We found that lean women (<24 kg/m^2) had a higher Σ_4DAP concentration than that of overweight/obese women (≥24 kg/m^2) (Σ_4DAP median: 243.84 vs. 219.15 nmol/g, $p = 0.001$, Mann-Whitney *U*-test), whereas older women (≥35 years of age) had lower Σ_4DAP concentrations in comparison with that of younger women (<35 years of age) (Σ_4DAP median: 197.23 vs. 242.20 nmol/g, $p = 0.02$, Mann-Whitney *U*-test).

The risk ratios (RR) and 95% confidence intervals (CI) for urinary DAP concentrations and clinical outcomes are presented in Table 4. Compared with women in the lowest quartile (Q_1) of DEP, women in the highest quartile (Q_4) had lower odds of successful implantation (adjusted RR = 0.69), clinical pregnancy (adjusted RR = 0.76; 95% CI: 0.62, 0.92), and live birth (adjusted RR = 0.79; 95% CI: 0.66, 0.96). There was a decreasing trend

Table 4. RR and 95% CI for preconception DAP concentrations and clinical outcomes among 522 women undergoing 785 IVF cycles.

		Implantation (273 women, 344 cycles)		Clinical pregnancy (237 women, 293 cycles)		Live birth (228 women, 282 cycles)	
Exposure	Women (cycles)	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
DMP (μg/g)							
Q1 (≤0.38)	131 (196)	Ref	Ref	Ref	Ref	Ref	Ref
Q2 (0.39–6.37)	130 (196)	1.09 (0.87, 1.37)	1.03 (0.83, 1.27)	1.02 (0.85, 1.24)	0.97 (0.81, 1.16)	1.03 (0.85, 1.24)	0.97 (0.81, 1.16)
Q3 (6.38–23.44)	131 (197)	0.94 (0.74, 1.20)	0.90 (0.72, 1.13)	0.98 (0.80, 1.20)	0.94 (0.78, 1.13)	0.96 (0.79, 1.17)	0.92 (0.77, 1.11)
Q4 (≥23.45)	130 (196)	0.92 (0.71, 1.19)	0.91 (0.71, 1.15)	0.96 (0.77, 1.19)	0.94 (0.77, 1.15)	0.99 (0.81, 1.22)	0.97 (0.80, 1.17)
p-trend	—	0.32	0.36	0.59	0.61	0.92	0.92
DMTP (μg/g)							
Q1 (≤0.79)	131 (201)	Ref	Ref	Ref	Ref	Ref	Ref
Q2 (0.80–1.44)	131 (197)	0.92 (0.73, 1.16)	0.83 (0.67, 1.03)	0.94 (0.76, 1.15)	0.85 (0.70, 1.02)	0.98 (0.80, 1.20)	0.89 (0.74, 1.07)
Q3 (1.45–2.50)	130 (190)	0.97 (0.77, 1.23)	0.94 (0.75, 1.16)	1.09 (0.89, 1.33)	1.03 (0.86, 1.24)	1.09 (0.89, 1.32)	1.04 (0.86, 1.24)
Q4 (≥2.51)	130 (197)	0.92 (0.72, 1.17)	0.87 (0.70, 1.09)	1.01 (0.82, 1.24)	0.94 (0.79, 1.13)	1.04 (0.85, 1.26)	0.97 (0.81, 1.16)
p-trend	—	0.63	0.48	0.75	0.96	0.66	0.91
DEP (μg/g)							
Q1 (≤1.15)	130 (196)	Ref	Ref	Ref	Ref	Ref	Ref
Q2 (1.16–9.76)	131 (196)	0.94 (0.76, 1.16)	0.89 (0.73, 1.08)	0.90 (0.76, 1.08)	0.88 (0.75, 1.03)	0.94 (0.79, 1.12)	0.92 (0.78, 1.08)
Q3 (9.77–41.30)	131 (197)	0.79 (0.62, 1.00)	0.80 (0.64, 0.98)	0.76 (0.62, 0.93)	0.78 (0.65, 0.93)	0.80 (0.65, 0.97)	0.81 (0.68, 0.97)
Q4 (≥41.31)	130 (196)	0.67 (0.52, 0.87)	0.69 (0.54, 0.87)	0.74 (0.60, 0.91)	0.76 (0.62, 0.92)	0.77 (0.63, 0.95)	0.79 (0.66, 0.96)
p-trend	—	0.004	0.005	0.02	0.02	0.02	0.04
DETP (μg/g)							
Q1 (≤1.01)	131 (199)	Ref	Ref	Ref	Ref	Ref	Ref
Q2 (1.02–1.85)	131 (195)	1.13 (0.91, 1.40)	1.10 (0.89, 1.35)	1.15 (0.95, 1.39)	1.11 (0.93, 1.33)	1.17 (0.97, 1.40)	1.13 (0.94, 1.35)
Q3 (1.86–3.55)	130 (197)	0.83 (0.64, 1.07)	0.85 (0.68, 1.08)	0.90 (0.72, 1.12)	0.91 (0.75, 1.11)	0.90 (0.72, 1.11)	0.90 (0.74, 1.09)
Q4 (≥3.56)	130 (194)	0.89 (0.69, 1.14)	0.89 (0.71, 1.11)	0.96 (0.78, 1.18)	0.96 (0.80, 1.16)	0.96 (0.78, 1.18)	0.96 (0.80, 1.16)
p-trend	—	0.19	0.19	0.38	0.45	0.35	0.40
Σ_4DAP (nmol/g)							
Q1 (≤58.75)	130 (197)	Ref	Ref	Ref	Ref	Ref	Ref
Q2 (58.76–240.70)	131 (195)	1.21 (0.98, 1.49)	1.08 (0.88, 1.32)	1.08 (0.90, 1.30)	0.99 (0.83, 1.17)	1.09 (0.91, 1.31)	1.01 (0.85, 1.19)
Q3 (240.71–592.02)	131 (197)	0.90 (0.70, 1.14)	0.83 (0.66, 1.05)	0.92 (0.75, 1.12)	0.86 (0.71, 1.04)	0.92 (0.76, 1.13)	0.87 (0.72, 1.05)
Q4 (≥592.03)	130 (196)	0.79 (0.60, 1.03)	0.77 (0.60, 0.99)	0.81 (0.65, 1.02)	0.80 (0.65, 0.99)	0.88 (0.71, 1.08)	0.86 (0.70, 1.05)
p-trend	—	0.01	0.02	0.02	0.03	0.09	0.10

Note: RR and 95% CI for preconception DAP concentrations and clinical outcomes were estimated using GEE models. —, no data; BMI, body mass index; CI, confidence interval; DAP, dialkylphosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GEE, generalized estimating equations; IVF, in vitro fertilization; PSS, perceived stress scale; RR, risk ratios.
[a]Adjusted for age, BMI, duration of infertility, smoking status, education, annual household income, infertility diagnosis, two items from the PSS-10 questionnaire ("Have you been upset because of something that happened unexpectedly?" and "Have felt unable to control the important things in your life?").

in the success of implantation, clinical pregnancy, and live birth with the increase in DEP metabolite concentrations (p-trends <0.05). The likelihood for successful implantation and clinical pregnancy was significantly lower, by 23% (adjusted RR = 0.77; 95% CI: 0.60, 0.99) and, by 20% respectively (adjusted RR = 0.80; 95% CI: 0.65, 0.99) for women in the Q_4 of Σ_4DAP (the sum of DMP, DMTP, DEP, and DETP) in comparison with the women with concentrations of the same in Q_1, with significant declining trends (p-trend <0.05) from the Q_1 to Q_4 concentrations. The likelihood of live birth was reduced by 14% for women in Q_4 compared with Q_1 of Σ_4DAP, with a suggestive downward trend (p-trend = 0.10, adjusted RR = 0.86; 95% CI: 0.70, 1.05). There were no significant associations between the other urinary DAP metabolites and clinical outcomes. In view of multiple testing of the associations between urinary DAP concentrations and clinical outcomes, the p-values in GEE models were adjusted for multiple comparisons using Benjamini-Hochberg correction to control FDR at <5%. p-Values for quartile-specific RR that were statistically significant in the primary analysis remained significant after adjusting for multiple comparisons (Table S5).

As shown in Table 5, there were no significant associations between urinary DAP concentrations and early IVF outcomes, including total and mature oocyte yields, endometrial wall thickness, peak estradiol levels, best embryo quality, or fertilization.

In addition, the results of the sensitivity analysis for the clinical outcomes were similar when restricted to the first cycle, as shown in Table S6. In the sensitivity analysis where we investigated whether individual DEP were primarily driving our results, we observed that except for individual DEP, there were no associations between urinary Σ_3DAP concentrations (the sum of DMP, DMTP, and DETP) and clinical outcomes (Table S7).

The analysis stratified by type of insemination found no association between the majority of the DAP metabolites concentrations and the proportion of MII oocytes or fertilization in either IVF or ICSI cycles (Table S8). Because the trends were similar, we combined the two groups (IVF and ICSI) to increase statistical power.

We also performed stratification analyses between DAP concentrations and clinical outcomes stratified by infertility cause (male or female factor groups). We observed similar associations in the female factor group, but no associations were found in the male factor group (Table S9).

In the analyses stratified by age, inverse associations of DEP and Σ_4DAP concentrations with clinical outcomes among older women (≥35 years of age) were stronger and had a more consistently monotonic trend than corresponding estimates for younger women (<35 years of age) (Table 6). However, none of the differences were significant (p-interaction for DEP and Σ_4DAP 0.44–0.82).

In the BMI-stratified analysis, associations of DEP with clinical pregnancy and live birth were similar between lean and overweight/obese women (p-interaction 0.32 and 0.34, respectively), whereas the inverse association between DEP and implantation was stronger for women with BMI <24 kg/m^2 than for heavier women (p-interaction = 0.09) (Table 7). There were no significant differences in associations between the clinical outcomes and Σ_4DAP or the other individual metabolites according to material BMI (p-interaction 0.14–0.98).

Discussion

This study assessed the association between environmental OP exposures and pregnancy outcomes in women with infertility who underwent IVF. We found that preconception OP exposure was associated with lower chance of successful implantation, clinical pregnancy, and live birth. We found no associations between urinary DAP concentrations and early IVF outcomes.

Several studies previously evaluated the effects of pesticide exposure on early IVF outcomes, with inconsistent results (Jirsová et al. 2010; Mahalingaiah et al. 2012; Al-Saleh et al. 2009; Al-Hussaini et al. 2018; Bloom et al. 2017). A study on organochlorines pesticides and reproductive health outcomes in 99 women who had IVF showed that the levels of 1,1,1-trichloro-2,2,-bis(4-chlorophenyl)ethane (DDT) in follicular fluid were negatively associated with the number of diploid oocytes (Jirsová et al. 2010). Another prospective study that examined the relationship between toxicants in reproductive fluid and IVF outcomes in 94 infertile couples found high concentrations of DDT, diazinon, and chlorpyrifos (CPF) (the most commonly used types of OP) in follicular fluid, and these were associated with lower number of oocytes retrieval (Al-Hussaini et al. 2018). In contrast, other studies reported no associations between pesticide exposures and early reproductive outcomes (Mahalingaiah et al. 2012; Al-Saleh et al. 2009). In our study, we did not observe associations between urinary DAP concentrations and early IVF outcomes. Different geographic areas and exposure scenarios of various populations, as well as different demographic characteristics (BMI, age, infertility diagnosis, and treatment protocols) might contribute to these conflicting results. However, most of the previous studies assessed a limited number of outcomes. Thus, our study brings additional evidence to support the effect of OP on reproduction because we report on comprehensive pregnancy outcomes following exposure to several OP metabolites.

In addition to evaluating associations between OP exposures and early IVF outcomes, we examined associations between urinary DAP concentrations and clinical outcomes. To our knowledge, the study most closely related to our is the EARTH Study, which enrolled 325 women who underwent 541 ART cycles and reported that regular consumption of fruits and vegetables with high pesticide residues was associated with lower probabilities of pregnancy and live birth (Chiu et al. 2018). However, the EARTH Study did not identify a specific pesticide or class of pesticides responsible for decreased female fertility but instead used the Pesticide Residue Burden Score (PRBS) and questionnaire on diet as a proxy measure of exposure to pesticides. In our study, we offer a quantitative estimate of DAP metabolites concentrations in urine as an objective measure of specific environmental OP exposures in women before conception.

In our study, we found detection rates for the measured DAP metabolites between 1.9% and 92%. Nevertheless, DAP metabolites concentrations in preconception women in our study (ΣDAP median 216.67 nmol/g) were much higher than those reported in developed countries such as the France (38.2 nmol/g) and the United States (102.80 nmol/g), although still comparable to those previously reported from Shanghai (198.43 nmol/g) and Jiangsu (295.80 nmol/g) provinces in China (Hu et al. 2018; Bradman et al. 2005; Liu et al. 2016; Debost-Legrand et al. 2016). The high urine DAP concentrations of OP in the current population may be attributable to heavy use and high residues in the common raw food supply in China. OP are the most widely used classes of insecticides in agricultural and residential areas in China, with 70,000 metric tons (approximately 154 million lb) of OP used in the year of 2015 (Shu et al. 2016). Although the use of OP for indoor and garden pest control was banned in the United States and several European countries in 2000 (Barr et al. 2010; Roca et al. 2014), OP are still commonly used in agriculture, accounting for 33% (20 million lb) of all insecticides used in the United States in 2012 (U.S. EPA 2017). A recent study from China has found that 32 pesticide residues, including CPF, dichlorvos, omethoate, methamidophos, parathion-methyl, parathion, and triazophos, were measured in 518 samples from 20 types of vegetables, with 7.7% of the detectable pesticide residues exceeding the national maximum residue

Table 5. The association between preconception DAP concentrations and early IVF outcomes among 522 women (785 IVF cycles).

	RR (95% CI)									
	DMP (μg/g)		DMTP (μg/g)		DEP (μg/g)		DETP (μg/g)		Σ_4DAP (nmol/g)	
Outcomes	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Fertilization, proportion										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	1.08 (0.86, 1.36)	1.15 (0.91, 1.46)	1.07 (0.87, 1.32)	1.21 (0.92, 1.59)	1.11 (0.90, 1.38)	1.17 (0.94, 1.46)	0.92 (0.72, 1.18)	0.95 (0.74, 1.23)	0.89 (0.79, 1.23)	1.04 (0.84, 1.30)
Q3[b]	1.14 (0.91, 1.41)	1.12 (0.90, 1.41)	0.96 (0.76, 1.22)	0.89 (0.70, 1.12)	1.07 (0.85, 1.33)	1.12 (0.89, 1.42)	0.97 (0.79, 1.18)	0.98 (0.79, 1.21)	1.01 (0.81, 1.26)	1.04 (0.82, 1.31)
Q4[b]	1.05 (0.84, 1.32)	1.01 (0.80, 1.29)	0.98 (0.79, 1.20)	0.92 (0.75, 1.14)	1.08 (0.87, 1.34)	1.08 (0.86, 1.36)	1.04 (0.87, 1.24)	1.05 (0.86, 1.27)	1.03 (0.84, 1.27)	1.00 (0.81, 1.25)
p-trend	0.58	0.95	0.63	0.15	0.56	0.62	0.59	0.62	0.70	0.95
Total oocytes, count										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	0.89 (0.78, 1.03)	0.93 (0.82, 1.06)	1.00 (0.86, 1.17)	1.00 (0.87, 1.15)	0.91 (0.77, 1.07)	0.97 (0.83, 1.13)	0.91 (0.78, 1.06)	0.90 (0.78, 1.03)	0.82 (0.71, 1.15)	0.88 (0.76, 1.02)
Q3[b]	0.93 (0.81, 1.08)	0.96 (0.84, 1.10)	0.97 (0.82, 1.14)	1.01 (0.86, 1.18)	0.99 (0.84, 1.16)	1.00 (0.86, 1.16)	1.02 (0.87, 1.19)	1.04 (0.89, 1.21)	1.01 (0.88, 1.16)	1.05 (0.92, 1.19)
Q4[b]	0.92 (0.79, 1.08)	0.97 (0.83, 1.12)	0.97 (0.83, 1.14)	0.99 (0.85, 1.14)	0.98 (0.83, 1.16)	0.99 (0.85, 1.15)	0.96 (0.82, 1.13)	0.98 (0.85, 1.14)	0.95 (0.82, 1.09)	0.98 (0.85, 1.12)
p-trend	0.40	0.700	0.69	0.87	0.88	0.98	0.93	0.81	0.99	0.77
Total MII oocytes, count										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	1.02 (0.82, 1.27)	1.07 (0.88, 1.30)	0.97 (0.78, 1.21)	0.97 (0.79, 1.18)	0.93 (0.74, 1.18)	1.01 (0.83, 1.24)	0.92 (0.73, 1.15)	0.91 (0.74, 1.11)	0.81 (0.69, 1.15)	1.08 (0.87, 1.34)
Q3[b]	0.97 (0.75, 1.25)	1.00 (0.81, 1.25)	0.93 (0.70, 1.24)	0.99 (0.77, 1.26)	1.05 (0.83, 1.33)	1.14 (0.92, 1.41)	0.95 (0.74, 1.22)	1.04 (0.82, 1.32)	1.02 (0.88, 1.18)	1.08 (0.87, 1.34)
Q4[b]	0.97 (0.75, 1.26)	1.02 (0.81, 1.28)	0.98 (0.76, 1.26)	1.00 (0.80, 1.24)	1.07 (0.81, 1.41)	1.17 (0.93, 1.46)	1.03 (0.79, 1.33)	1.02 (0.82, 1.27)	0.94 (0.81, 1.09)	0.96 (0.78, 1.18)
p-trend	0.73	0.98	0.81	0.98	0.49	0.11	0.88	0.64	0.98	0.73
Best-quality embryos, count										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	0.97 (0.80, 1.18)	1.02 (0.85, 1.23)	1.01 (0.83, 1.23)	1.04 (0.87, 1.26)	1.01 (0.83, 1.24)	0.96 (0.78, 1.19)	0.91 (0.74, 1.11)	0.89 (0.73, 1.09)	0.87 (0.74, 1.02)	0.83 (0.67, 1.02)
Q3[b]	0.96 (0.78, 1.17)	0.99 (0.82, 1.19)	0.94 (0.76, 1.18)	1.00 (0.81, 1.22)	1.14 (0.92, 1.41)	1.12 (0.91, 1.40)	1.04 (0.82, 1.32)	1.07 (0.87, 1.31)	1.06 (0.92, 1.22)	1.02 (0.84, 1.24)
Q4[b]	1.02 (0.82, 1.27)	1.08 (0.88, 1.32)	0.96 (0.78, 1.18)	0.97 (0.80, 1.18)	1.17 (0.93, 1.46)	1.13 (0.92, 1.40)	1.02 (0.82, 1.27)	1.00 (0.80, 1.22)	0.97 (0.84, 1.13)	1.00 (0.81, 1.22)
p-trend	0.92	0.55	0.55	0.67	0.11	0.11	0.64	0.61	0.73	0.64
E_2 trigger levels, pmol/L										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	1.02 (0.94, 1.11)	1.04 (0.96, 1.13)	1.04 (0.96, 1.13)	1.04 (0.97, 1.12)	0.97 (0.88, 1.06)	0.99 (0.91, 1.08)	1.05 (0.96, 1.14)	1.05 (0.97, 1.13)	0.96 (0.88, 1.05)	1.01 (0.93, 1.10)
Q3[b]	1.00 (0.92, 1.08)	1.02 (0.95, 1.10)	1.01 (0.93, 1.10)	1.03 (0.95, 1.13)	1.01 (0.92, 1.10)	1.02 (0.94, 1.10)	1.09 (1.00, 1.19)	1.10 (1.01, 1.19)	1.03 (0.95, 1.12)	1.06 (0.98, 1.14)
Q4[b]	1.01 (0.93, 1.10)	1.03 (0.96, 1.12)	1.04 (0.96, 1.12)	1.04 (0.96, 1.12)	1.01 (0.93, 1.10)	1.02 (0.94, 1.10)	1.03 (0.95, 1.12)	1.04 (0.96, 1.12)	1.02 (0.94, 1.10)	1.04 (0.96, 1.12)
p-trend	0.97	0.51	0.53	0.39	0.51	0.56	0.29	0.17	0.38	0.21
Endometrial wall thickness (mm)										
Q1[b]	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Q2[b]	0.97 (0.55, 1.71)	0.99 (0.59, 1.71)	1.00 (0.57, 1.75)	1.18 (0.69, 1.84)	1.36 (0.77, 2.38)	1.49 (0.89, 2.52)	1.09 (0.63, 1.90)	0.99 (0.58, 1.69)	0.50 (0.29, 0.88)	0.60 (0.35, 1.03)
Q3[b]	0.85 (0.49, 1.49)	0.90 (0.53, 1.53)	0.78 (0.46, 1.32)	0.82 (0.50, 1.36)	1.16 (0.66, 2.04)	1.16 (0.68, 1.97)	0.78 (0.45, 1.35)	0.77 (0.45, 1.30)	0.89 (0.51, 1.55)	0.92 (0.54, 1.58)
Q4[b]	0.61 (0.34, 1.08)	0.65 (0.38, 1.13)	0.99 (0.56, 1.78)	1.04 (0.62, 1.78)	1.23 (0.69, 2.20)	1.27 (0.74, 2.19)	0.80 (0.44, 1.44)	0.82 (0.47, 1.41)	0.60 (0.35, 1.05)	0.67 (0.40, 1.15)
p-trend	0.09	0.13	0.79	0.85	0.64	0.62	0.29	0.33	0.25	0.35

Note: Associations between preconception DAP concentrations and early IVF outcomes were estimated using GEE models. BMI, body mass index; CI, confidence interval; DAP, dialkylphosphate; DEP, diethylphosphate; DETP; diethylthiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GEE, generalized estimating equations; IVF, *in vitro* fertilization; PSS, Perceived Stress Scale; RR, risk ratios; PSS, Perceived Stress Scale; RR, risk ratio.

[a]Adjusted for age, BMI, duration of infertility, smoking status, education, annual household income, infertility diagnosis, two items from the PSS-10 questionnaire ("Have you been upset because of something that happened unexpectedly?" and "Have felt unable to control the important things in your life?").

[b]The cut points of DAP metabolites were the same as the cut points shown in Table 4.

Table 6. Adjusted RR (95% CI) for DAP concentrations and clinical outcomes stratified by age among 522 women (785 IVF cycles).

	Adjusted RR (95% CI)[a]											
	Implantation				Clinical pregnancy				Live birth			
Exposure	<35 years (196 women, 236 cycles)		≥35 years (77 women, 108 cycles)		<35 years (173 women, 207 cycles)		≥35 years (64 women, 86 cycles)		<35 years (167 women, 201 cycles)		≥35 years (61 women, 81 cycles)	
DMP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	1.32 (0.89, 1.95)	0.16	0.88 (0.69, 1.12)	0.30	1.13 (0.82, 1.56)	0.46	0.91 (0.74, 1.11)	0.34	1.10 (0.80, 1.51)	0.55	0.90 (0.73, 1.10)	0.29
Q3[b]	1.20 (0.79, 1.81)	0.39	0.88 (0.69, 1.12)	0.30	1.10 (0.79, 1.53)	0.58	0.94 (0.76, 1.15)	0.53	1.09 (0.79, 1.51)	0.61	0.96 (0.79, 1.17)	0.67
Q4[b]	0.93 (0.59, 1.47)	0.76	0.80 (0.60, 1.05)	0.11	0.95 (0.65, 1.38)	0.77	0.89 (0.70, 1.11)	0.30	0.96 (0.67, 1.40)	0.83	0.94 (0.76, 1.16)	0.56
p-trend	0.75	—	0.13	—	0.81	—	0.37	—	0.88	—	0.71	—
p-interaction	0.56		—		0.79		—		0.90		—	
DMTP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	0.86 (0.58, 1.27)	0.43	0.75 (0.59, 0.95)	0.02	0.85 (0.60, 1.19)	0.34	0.80 (0.65, 0.99)	0.04	0.87 (0.62, 1.22)	0.41	0.88 (0.72, 1.07)	0.20
Q3[b]	1.16 (0.79, 1.69)	0.45	0.80 (0.63, 1.01)	0.06	1.23 (0.90, 1.69)	0.19	0.93 (0.76, 1.13)	0.47	1.19 (0.88, 1.63)	0.26	0.93 (0.77, 1.13)	0.47
Q4[b]	0.87 (0.58, 1.30)	0.51	0.91 (0.71, 1.15)	0.41	0.96 (0.70, 1.33)	0.81	0.98 (0.80, 1.20)	0.82	0.98 (0.72, 1.35)	0.91	0.99 (0.82, 1.20)	0.91
p-trend	0.85	—	0.56	—	0.66	—	0.91	—	0.57	—	0.98	—
p-interaction	0.12		—		0.41		—		0.45		—	
DEP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	0.93 (0.64, 1.35)	0.71	0.90 (0.72, 1.12)	0.35	0.92 (0.67, 1.25)	0.60	0.91 (0.76, 1.09)	0.29	0.95 (0.70, 1.29)	0.73	0.93 (0.79, 1.11)	0.44
Q3[b]	0.83 (0.56, 1.22)	0.35	0.75 (0.59, 0.97)	0.03	0.81 (0.58, 1.12)	0.20	0.76 (.62, 0.93)	0.01	0.85 (0.61, 1.17)	0.32	0.77 (0.64, 0.94)	0.01
Q4[b]	0.76 (0.49, 1.17)	0.21	0.68 (0.53, 0.88)	0.003	0.88 (0.62, 1.24)	0.45	0.72 (0.59, 0.89)	0.002	0.91 (0.65, 1.28)	0.58	0.76 (0.62, 0.92)	0.01
p-trend	0.19	—	0.001	—	0.33	—	0.001	—	0.45	—	0.002	—
p-interaction	0.82		—		0.44		—		0.48		—	
DETP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	1.47 (1.02, 2.12)	0.05	0.77 (0.61, 0.98)	0.04	1.30 (0.95, 1.77)	0.11	0.90 (0.74, 1.09)	0.29	1.36 (1.00, 1.84)	0.05	0.89 (0.74, 1.08)	0.24
Q3[b]	0.86 (0.55, 1.34)	0.50	0.74 (0.57, 0.96)	0.02	0.92 (0.63, 1.33)	0.65	0.82 (0.66, 1.02)	0.08	0.88 (0.61, 1.27)	0.59	0.81 (0.66, 1.01)	0.05
Q4[b]	0.95 (0.62, 1.47)	0.82	0.83 (0.66, 1.05)	0.12	1.05 (0.75, 1.49)	0.76	0.87 (0.70, 1.07)	0.18	1.07 (0.76, 1.50)	0.70	0.86 (0.70, 1.04)	0.13
p-trend	0.29	—	0.12	—	0.75	—	0.14	—	0.72	—	0.09	—
p-interaction	0.32		—		0.40		—		0.35		—	
Σ_4DAP (nmol/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	1.12 (0.78, 1.60)	0.54	0.98 (0.77, 1.25)	0.90	0.94 (0.70, 1.26)	0.68	0.98 (0.80, 1.20)	0.83	0.98 (0.73, 1.32)	0.90	0.98 (0.81, 120)	0.86
Q3[b]	0.76 (0.49, 1.17)	0.21	0.88 (0.68, 1.14)	0.33	0.81 (0.58, 1.14)	0.23	0.89 (0.72, 1.10)	0.27	0.85 (0.60, 1.19)	0.34	0.91 (0.75, 1.11)	0.35
Q4[b]	0.89 (0.58, 1.38)	0.61	0.69 (0.51, 0.93)	0.02	0.86 (0.60, 1.23)	0.42	0.76 (0.60, 0.97)	0.03	0.91 (0.64, 1.29)	0.60	0.81 (0.64, 1.01)	0.06
p-trend	0.25	—	0.002	—	0.26	—	0.003	—	0.43	—	0.01	—
p-interaction	0.68		—		0.78		—		0.82		—	

Note: —, no data; BMI, body mass index; DAP, dialkylphosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GEE, generalized estimating equations; IVF, *in vitro* fertilization; PSS, Perceived Stress Scale; RR, risk ratios.

[a]Adjusted for BMI, duration of infertility, smoking status, education, annual household income, infertility diagnosis, two items from the PSS-10 questionnaire ("Have you been upset because of something that happened unexpectedly?" and "Have felt unable to control the important things in your life?").

[b]The cut point of DAP metabolites for younger women (<35 y): DMP (μg/g): Q1 (≤0.30), Q2 (0.31–4.94), Q3 (4.95–18.84), Q4 (≥18.85); DMTP (μg/g): Q1 (≤0.73), Q2 (0.74–1.44), Q3 (1.45–2.47), Q4 (≥2.48); DEP (μg/g): Q1 (≤1.50), Q2 (1.51–11.72), Q3 (11.73–45.41), Q4 (≥45.42); DETP (μg/g): Q1 (≤0.99), Q2 (1.00–1.85), Q3 (1.86–3.56), Q4 (≥3.57); Σ_4DAP (nmol/g): Q1 (≤45.35), Q2 (45.36–242.20), Q3 (242.21–655.12), Q4 (≥655.13).
The cut point for older women (≥35 y): DMP (μg/g): Q1 (≤0.77), Q2 (0.78–9.05), Q3 (9.06–27.38), Q4 (≥27.39); DMTP (μg/g): Q1 (≤0.81), Q2 (0.82–1.40), Q3 (1.41–2.62), Q4 (≥2.63); DEP (μg/g): Q1 (≤0.69), Q2 (0.70–8.68), Q3 (8.69–36.29), Q4 (≥36.30); DETP (μg/g): Q1 (≤1.04), Q2 (1.05–1.85), Q3 (1.86–3.25), Q4 (≥3.26); Σ_4DAP (nmol/g): Q1 (≤74.29), Q2 (74.30–236.13), Q3 (236.14–538.34), Q4 (≥538.35).

Table 7. Adjusted RR (95% CI) for DAP concentrations and clinical outcomes stratified by BMI among 522 women (785 IVF cycles).

	Adjusted RR (95% CI)[a]											
	Implantation				Clinical pregnancy				Live birth			
Exposure	<24 kg/m^2 (218 women, 272 cycles)		≥24 kg/m^2 (55 women, 72 cycles)		<24 kg/m^2 (190 women, 232 cycles)		≥24 kg/m^2 (47 women, 61 cycles)		<24 kg/m^2 (182 women, 222 cycles)		≥24 kg/m^2 (46 women, 60 cycles)	
DMP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	1.08 (0.84, 1.38)	0.55	0.80 (0.55, 1.15)	0.22	1.05 (0.85, 1.29)	0.67	0.89 (0.63, 1.25)	0.49	1.04 (0.84, 1.27)	0.74	0.93 (0.67, 1.29)	0.66
Q3[b]	0.92 (0.70, 1.21)	0.53	0.90 (0.64, 1.26)	0.54	0.91 (0.72, 1.14)	0.42	1.01 (0.73, 1.40)	0.95	0.90 (0.72, 1.13)	0.37	1.02 (0.74, 1.40)	0.90
Q4[b]	1.01 (0.76, 1.33)	0.95	0.57 (0.38, 0.83)	0.004	1.06 (0.84, 1.33)	0.64	0.67 (0.46, 0.99)	0.05	1.11 (0.89, 1.39)	0.35	0.69 (0.47, 1.00)	0.06
p-trend	0.78	—	0.01	—	0.94	—	0.07	—	0.60	—	0.07	—
p-interaction	0.14		—		0.27		—		0.19		—	
DMTP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	0.84 (0.64, 1.08)	0.17	1.16 (0.83, 1.64)	0.39	0.86 (0.68, 1.09)	0.22	1.05 (0.75, 1.46)	0.78	0.91 (0.73, 1.15)	0.44	1.08 (0.78, 1.50)	0.63
Q3[b]	0.95 (0.73, 1.22)	0.66	0.86 (0.61, 1.21)	0.39	1.04 (0.84, 1.29)	0.73	1.04 (0.78, 1.39)	0.77	1.03 (0.83, 1.27)	0.80	1.05 (0.79, 1.40)	0.72
Q4[b]	0.81 (0.62, 1.05)	0.11	1.00 (0.68, 1.48)	0.99	0.91 (0.73, 1.14)	0.42	0.95 (0.67, 1.35)	0.77	0.95 (0.77, 1.18)	0.65	0.95 (0.68, 1.34)	0.77
p-trend	0.22	—	0.60	—	0.78	—	0.81	—	0.90	—	0.79	—
p-interaction	0.76		—		0.97		—		0.97		—	
DEP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	0.88 (0.70, 1.12)	0.32	0.99 (0.77, 1.33)	0.94	0.90 (0.74, 1.09)	0.28	0.96 (0.75, 1.25)	0.78	0.94 (0.77, 1.14)	0.51	0.97 (0.76, 1.25)	0.83
Q3[b]	0.74 (0.57, 0.94)	0.02	0.95 (0.68, 1.33)	0.78	0.75 (0.61, 0.94)	0.01	0.81 (0.60, 1.11)	0.19	0.78 (0.63, 0.96)	0.02	0.85 (0.63, 1.14)	0.27
Q4[b]	0.70 (0.53, 0.93)	0.01	0.77 (0.61, 1.00)	0.05	0.77 (0.61, 0.98)	0.03	0.77 (0.60, 1.01)	0.05	0.82 (0.66, 1.04)	0.09	0.77 (0.60, 1.06)	0.06
p-trend	0.004	—	0.08	—	0.01	—	0.05	—	0.05	—	0.08	—
p-interaction	0.09		—		0.32		—		0.34		—	
DETP (μg/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	1.10 (0.86, 1.41)	0.44	1.11 (0.77, 1.61)	0.57	1.13 (0.92, 1.39)	0.25	1.13 (0.79, 1.61)	0.52	1.15 (0.93, 1.41)	0.20	1.17 (0.83, 1.66)	0.38
Q3[b]	0.90 (0.68, 1.18)	0.45	0.82 (0.57, 1.18)	0.29	0.92 (0.73, 1.17)	0.51	0.77 (0.52, 1.14)	0.19	0.92 (0.73, 1.15)	0.46	0.78 (0.52, 1.15)	0.20
Q4[b]	0.86 (0.66, 1.12)	0.25	0.90 (0.63, 1.28)	0.56	0.91 (0.72, 1.14)	0.39	1.01 (0.72, 1.41)	0.96	0.92 (0.74, 1.15)	0.45	1.02 (0.73, 1.43)	0.89
p-trend	0.13	—	0.31	—	0.18	—	0.59	—	0.19	—	0.60	—
p-interaction	0.98		—		0.52		—		0.34		—	
Σ_4DAP (nmol/g)												
Q1[b]	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value	Ref	*p*-value
Q2[b]	0.98 (0.78, 1.24)	0.88	1.15 (0.85, 1.56)	0.35	0.90 (0.73, 1.10)	0.29	1.05 (0.78, 1.41)	0.75	0.89 (0.73, 1.09)	0.26	1.09 (0.83, 1.45)	0.53
Q3[b]	0.71 (0.54, 0.93)	0.01	0.94 (0.70, 1.26)	0.69	0.73 (0.58, 0.92)	0.008	0.90 (0.70, 1.17)	0.43	0.77 (0.61, 0.96)	0.02	0.91 (0.70, 1.17)	0.44
Q4[b]	0.83 (0.63, 1.11)	0.21	0.70 (0.45, 1.07)	0.10	0.86 (0.68, 1.09)	0.22	0.71 (0.49, 1.04)	0.08	0.91 (0.73, 1.14)	0.41	0.71 (0.50, 1.03)	0.07
p-trend	0.06	—	0.06	—	0.09	—	0.05	—	0.25	—	0.06	—
p-interaction	0.80		—		0.45		—		0.58		—	

Note: —, no data; BMI, body mass index; DAP, dialkylphosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; GEE, generalized estimating equations; IVF, *in vitro* fertilization; PSS, Perceived Stress Scale; RR, risk ratios.

[a]Adjusted for age, duration of infertility, smoking status, education, annual household income, infertility diagnosis, two items from the PSS-10 questionnaire ("Have you been upset because of something that happened unexpectedly?" and "Have felt unable to control the important things in your life?").

[b]The cut point of DAP metabolites for lean women (<24 kg/m^2): DMP (μg/g): Q1 (≤0.33), Q2 (0.34–7.03), Q3 (7.04–27.13), Q4 (≥27.14); DMTP (μg/g): Q1 (≤0.81), Q2 (0.82–1.51), Q3 (1.52–2.65), Q4 (≥2.66); DEP (μg/g): Q1 (≤1.01), Q2 (1.02–9.05), Q3 (9.06– 42.90), Q4 (≥42.91); DETP (μg/g): Q1 (≤1.04), Q2 (1.05–1.88), Q3 (1.89–3.56), Q4 (≥3.57); Σ_4DAP (nmol/g): Q1 (≤52.23), Q2 (52.24–243.84), Q3 (243.85–669.93), Q4 (≥669.94).
The cut point for overweight/obese women (≥24 kg/m^2): DMP (μg/g): Q1 (≤0.96), Q2 (0.97–6.35), Q3 (6.36–14.19), Q4 (≥14.20); DMTP (μg/g): Q1 (≤0.69), Q2 (0.70–1.19), Q3 (1.20–2.20), Q4 (≥2.21); DEP (μg/g): Q1 (≤0.69), Q2 (0.70–1.19), Q3 (1.20–36.68), Q4 (≥36.69); DETP (μg/g): Q1 (≤1.00), Q2 (1.01–1.84), Q3 (1.85–3.32), Q4 (≥3.33); Σ_4DAP (nmol/g): Q1 (≤73.00), Q2 (73.01–219.15), Q3 (219.16–423.96), Q4 (≥423.97).

limits (MRL) (0.01 mg/kg for triazophos, 0.05 mg/kg for CPF and parathion, and 0.02 mg/kg for the other four OP listed above) (Yan et al. 2017). According to the U.S. Department of Agriculture Pesticide Data Program, in 2016, 85% of fruits and vegetables (FV) in U.S. markets had detectable pesticide residues; however, the detectable residues in most FV (>99%) did not exceed tolerance levels (USDA). Collectively, the results from these studies suggest the presence of pesticides in diets globally. Such pesticides are products that potentially cause adverse effects on reproduction and exposure to them should be carefully considered in women undergoing IVF treatments.

Experimental evidence of OP-induced alterations of reproductive function might contribute to explaining our findings. The mechanisms of OP toxicity, such as oxidative stress, apoptosis, genotoxicity, and cell cycle disturbance, may occur from the time of early embryo development to birth (Jin et al. 2015; Wang et al. 2017a; Rahman et al. 2020). It is possible that reproductive damages are accumulating during reproductive cycles. Molecular changes associated with preconception OP exposures might be too subtle to be detected morphologically at the blastocyst stage but later impair pregnancy maintenance and survival (Deng et al. 2020). Another proposed potential mechanism was that male environmental exposures may have significant adverse effects on the early IVF outcomes. Carignan et al. (2018) studied 201 couples with IVF treatments in Boston, Massachusetts, and reported that paternal preconception exposure to TDCIPP [a type of organophosphate flame retardant (FR)] had an adverse impact on successful oocyte fertilization, whereas female preconception exposure to ΣPFR was more relevant to later clinical adverse outcomes. Therefore, we could infer that the unmeasured male OP exposures could have potentially affected our results. Thus, it can be speculated that OP may affect both reproductive function and pregnancy outcomes through a more complex and complicated gender-interrelated mechanism. Additional studies are needed to elucidate the underlying mechanisms of action of OP more precisely.

Our results are supported by other experimental data. Studies in mouse embryos observed a dose-dependent inhibitory effect of pesticide on implantation as well as a significant decrease in the number of live pups (Cavieres et al. 2002; Tian and Yamauchi 2003; Zhou et al. 2018). Another study in porcine ovarian cells demonstrated that both malathion and diazinon decrease the efficiency of *in vitro* maturation of porcine oocytes and damage early embryo development (Ducolomb et al. 2009). More recently, evidence of OP developmental toxicity in zebrafish suggested various teratogenic effects and increased death of embryos (Pamanji et al. 2015).

In our study, DEP was the dominant individual metabolite, consistent with previous studies in China (Hu et al. 2018; Wang et al. 2017b), and was primarily responsible for associations between the summed DAP metabolites and clinical outcomes. Several factors may explain this phenomenon. First, the difference of metabolite profiles might be due to a different pesticide use pattern in China in contrast with patterns in other countries like the United States. CPF, a typical diethylphosphate, is widely used as an insecticide in agriculture, accounting for 68% of the total current-use pesticides in China (Li et al. 2014). The extensive application of CPF has resulted in environmental contamination, and it has been detected frequently in FV; 3,5,6-trichloro-2-pyridinol (TCPY), a metabolite of CPF, was detected in human urine and blood (Li et al. 2019; Liu et al. 2014). Second, once entering the human body, OP can be enzymatically converted to their highly toxic oxon forms, which then react with available body cholinesterase. The oxon can also be enzymatically or spontaneously hydrolyzed to form a DAP metabolite and another specific metabolite moiety (Barr and Angerer 2006). For example, CPF can be metabolized to DEP form and TCPY. If a pesticide is not converted to its oxon form, it can undergo a hydrolysis to its organic group metabolite and dialkylthionate metabolites. For CPF, these metabolites are DETP and TCPY (Wessels et al. 2003). We speculated that OP may be enzymatically converted preponderantly to the more toxic oxon, which in turn is metabolized to DEP metabolite. This potential biological mechanism lends more support to our findings.

Associations between preconception OP exposures (as indicated by urinary DEP metabolites) and clinical outcomes were somewhat stronger among older women than younger women, suggesting that associations between OP exposures and pregnancy outcomes may differ by age. However, the differences were not significant and should be interpreted with caution. Several previous studies have reported evidence of age-related differences in the impact of environmental contaminant exposures on female fecundability (Wang et al. 2018; Rattan et al. 2017). Hu et al. (2018) suggested that female reproductive function may be more susceptible to the adverse effects of OP exposures at an older age (≥32 years of age). Subsequently, several other studies also showed that pesticide exposures may aggravate the deterioration of embryonic development by disrupting the homeostasis of reproductive hormones in older women (Yilmaz et al. 2020; Lauria et al. 2006).

In regard to the biological plausibility of our findings, we found that lean women have higher DAP metabolites (Σ_4DAP median: 243.84 nmol/g vs. 219.15 nmol/g) than overweight/obese women, which was consistent with the results from previous studies (Llop et al. 2017; Van den Dries et al. 2018). We could infer that lean women tend to have a healthier lifestyle, with more FV in their diet, which may result in higher DAP concentrations. It is well-documented that women's weight has implications for infertility and reproduction, and the natural history of reproductive disorders may not be the same in lean women in comparison with overweight/obese women (Davies 2006). We estimated stronger associations between DEP and implantation in lean women compared with overweight/obese women, but other associations were similar between groups, and there were relatively few overweight/obese women in our study. Additional studies on larger populations are warranted.

Our study has several strengths. First, to our knowledge, this study has the largest sample size and systematic exploration into the pregnancy outcomes among Chinese women with artificial conception. The ART conception enabled us to observe the pregnancy outcomes ranging chronologically from oocyte retrieval, oocyte fertilization, embryo quality, and the rate of fertilization and implantation, to clinical pregnancy and live birth, reproductive events that are not observable in women who conceive spontaneously (Messerlian et al. 2016). Second, the prospective study design allowed comprehensive and contemporaneous retrieval of information about pregnancy outcomes from questionnaires with little recall bias (Yilmaz et al. 2020). Third, we were able to quantify a wide range of OP metabolites and to evaluate the associations between the concentrations of these metabolites and IVF outcomes.

A potential limitation of our study is that our findings may not be generalizable to the population of women who conceive spontaneously. First, it is possible that women undergoing IVF are more sensitive to OP exposures for a variety of reasons, including their causal history of infertility. Nevertheless, the prevalence of infertility in China is approximately 15%–20%, suggesting that our results may still be applicable to a large portion of the general population (Qiao and Feng 2014).

Second, although urinary concentrations of DAP metabolites may reflect a person's exposure to both parents' OP compounds

and the potentially low toxic preformed metabolites in the environment, current methodology cannot assess the provenience but rather characterizes and integrates multiple OP exposures from different sources (Clune et al. 2012). Measurement of pesticides in blood may more accurately reflect the dose to the target organ than measurement of metabolites in urine (Needham et al. 1995; Perera et al. 2003). Although blood measurements may be preferable in certain cases, estimating OP exposure with urinary concentrations of DAP metabolites has an important advantage beyond the ease of specimen collection (Eskenazi et al. 2004). The DAP metabolites reflect exposure to approximately 80% of OP, although a small number of OP (e.g., acephate) do not devolve into these urinary metabolites (Eskenazi et al. 2004). On the other hand, DAPs are nonspecific, and measurement of DAP metabolites does not allow differentiation between exposures from more or less toxic pesticides. Not all OP that devolve to the same metabolite are equally toxic; for example, both oxydemeton-methyl and malathion devolve to DMP metabolites, but the former is much more toxic (Eskenazi et al. 2007). Thus, although measurement of DAP metabolites is a nonspecific measure, it does offer an integrated measure of exposure to a class of pesticides.

Third, we measured urinary DAP metabolites at a single time point during the study: at enrollment. We are limited in our ability to assess what the average cumulative dose from different sources was and to what extent these measurements accurately reflected exposure throughout the entire critical period of reproduction. Collecting single spot urine samples might be less-representative biomarkers of chronic OP exposure because OP have a relatively short elimination half-life (12–36 h) and high within-person variability (Bradman et al. 2013; Spaan et al. 2015). However, a previous study showed that a spot urine sample had moderate sensitivity for predicting an individual's longer-term exposure over several weeks or months. Nonetheless considering the habitual lifestyle and the daily exposure to same microenvironment over the course of weeks or months for any given individual, it can be assumed that single spot urine sample does reflect with a high degree of accuracy the OP exposure of that individual (Meeker et al. 2005).

Fourth, in light of multiple comparison statistical procedures used in this study, false positive findings cannot be fully ruled out. However, we have used statistical procedures to control for multiple comparisons, and our findings were found significant or marginally significant using a stringent cutoff of 0.05 after adjustment for multiple tests, thus limiting the chance of false positive results. Fifth, we did not consider the male partner's exposure, which may be correlated with his female partner and could contribute and bias the observed association. Last, it is possible that our study results are influenced by uncontrolled confounding provided by unconsidered and possibly important environmental reproductive toxicants, such as phthalate, bisphenol A, and organophosphate FR, which have also been reported to affect IVF outcomes (Messerlian et al. 2016; Ehrlich et al. 2012; Mok-Lin et al. 2010; Messerlian et al. 2018). More research in this area is needed to shed more light into the complex interaction between environmental exposure and reproductive outcomes.

Conclusions

Our study contributes evidence that preconception environmental exposure to OP has adverse clinical outcomes in IVF pregnancies. Future research should also interrogate pesticide mixture exposures and explore the potential effect of OP on male and couple's reproductive health.

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